For general laboratory use.

High Pure FFPE RNA Micro Kit

Version: 10
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For the isolation of total RNA from formalin-fixed, paraffin-embedded tissue.

Cat. No. 04 823 125 001 1 kit
50 isolations

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial	Cap	Label	Function / Description	Content
1	white	High Pure FFPE RNA Micro Kit, Tissue Lysis Buffer	▪ Ready-to-use solution. ▪ For the lysis of cells.	1 bottle, 20 ml
2	pink	High Pure FFPE RNA Micro Kit, Proteinase K, recombinant, PCR grade	▪ Lyophilized ▪ For sample homogenization and inactivation of endogenous nucleases.	1 vial, 100 mg
3	green	High Pure FFPE RNA Micro Kit, Binding Buffer	▪ Contains 5 M guanidine thiocyanate. ▪ Ready-to-use solution.	1 bottle, 80 ml
4	black	High Pure FFPE RNA Micro Kit, Wash Buffer I	▪ Contains 5 M guanidine-HCl (final concentration after addition of ethanol). ▪ For RNA isolation.	1 vial, 33 ml
5	blue	High Pure FFPE RNA Micro Kit, Wash Buffer II	For RNA isolation.	1 vial, 10 ml
6	white	High Pure FFPE RNA Micro Kit, DNase I	▪ Lyophilized ▪ For digestion of residual DNA.	1 vial, 4 kU
7	colorless	High Pure FFPE RNA Micro Kit, DNase Incubation Buffer, 10x conc.	Ready-to-use solution.	1 vial, 1 ml
8	colorless	High Pure FFPE RNA Micro Kit, Elution Buffer	▪ Water, PCR Grade ▪ Ready-to-use solution.	1 bottle, 30 ml
9	–	High Pure FFPE RNA Micro Kit, High Pure Micro Filter Tubes	For use of up to 500 µl sample volume.	5 bags, 10 polypropylene filter tubes each
10	–	High Pure FFPE RNA Micro Kit, Collection Tubes	For RNA isolation.	2 bags, 50, 2 ml polypropylene tubes each

⚠ ***All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a +37°C water bath until the precipitates have dissolved.***

i The buffers can show a slight yellow color. This will have no impact on the function of the buffer.

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	white	Tissue Lysis Buffer	Store at +15 to +25°C. ⚠ ***Storage at +2 to +8°C or −15 to −25°C will adversely impact nucleic acid purification due to the formation of precipitates in the solutions.***
2	pink	Proteinase K, recombinant, PCR grade	
3	green	Binding Buffer	
4	black	Wash Buffer I	
5	blue	Wash Buffer II	
6	white	DNase I	
7	colorless	DNase Incubation Buffer, 10x conc.	
8	colorless	Elution Buffer	
9	–	High Pure Micro Filter Tubes	Store at +15 to +25°C.
10	–	Collection Tubes	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment and reagents

- Absolute ethanol
- 70% ethanol
- Hemo-De or Xylene
- 10% SDS
- Sterile microcentrifuge tubes, 1.5 or 2.0 ml
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force

1.4. Application

The High Pure FFPE RNA Micro Kit is designed for the isolation of total RNA from formalin-fixed, paraffin-embedded tissue samples for use in RT-PCR. The quality of RNA from paraffin-sections achieved with the kit is suitable for the relative quantification of mRNA with RT-PCR, especially on the LightCycler® 2.0 System.

1.5. Preparation Time

Assay Time

Total time

Approximately 30 minutes, without Proteinase K incubation and deparaffinization.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The High Pure FFPE RNA Micro Kit can be used with 1 to 10 µm sections from formalin-fixed, paraffin-embedded mammalian tissues, including human research samples:

- Colon
- Breast
- Liver
- Kidney
- Spleen

⚠ ***Section thickness as well as yield and quality of the isolated RNA are strongly related to type of tissue, age of sample, as well as fixation protocol used.***

Control Reactions

ⓘ *It is the user's responsibility to apply an appropriate control concept.*

General Considerations

Precautions

- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wash hands thoroughly after handling samples and reagents.
- Wear protective disposable gloves, laboratory coats, and eye protection when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use only sterile disposable polypropylene tubes, disposable pipettes, and nuclease-free pipette tips to avoid RNase contamination.
  ⓘ *Use general precautions described in the literature.*
- Handle all samples as if potentially infectious, using safe laboratory procedures.
  ⓘ *As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.*
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, finish
- PCR/RT-PCR sample preparation before starting PCR/RT-PCR setup. Perform sample preparation, PCR/RT-PCR setup, and the PCR/RT-PCR run itself in separate locations.
- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.

Handling samples

Samples can also be stored at −80°C in Lysis/Binding Buffer after disruption and homogenization. Yields may vary depending on storage time. Intracellular RNases become inactivated during fixation. Therefore, formalin-fixed, paraffin-embedded tissue can be stored and handled at room temperature.

ⓘ *Formalin-fixation might cause RNA degradation and crosslinking to proteins.*

Safety Information

Binding Buffer and Wash Buffer I contain guanidine hydrochloride which is an irritant.

- Always wear gloves and follow standard safety precautions to minimize contact when handling.
- Do not allow buffers to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns.
- If you spill the reagents, dilute the spill with water before wiping it up.
- Never store or use the Binding Buffer near human or animal food.

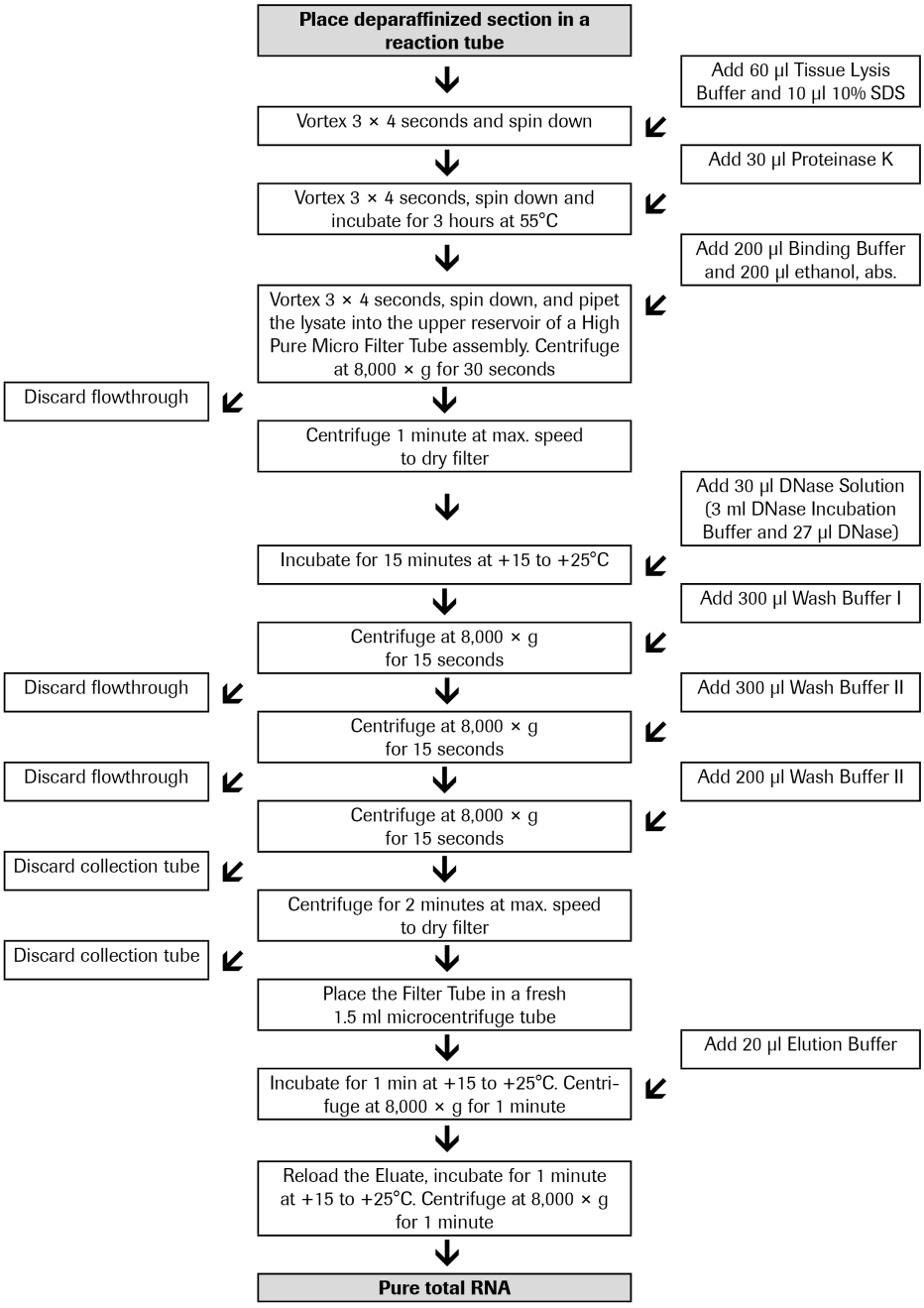
Do not allow the Lysis Buffers to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.

Working Solution

Content	Reconstitution / Preparation	Storage and Stability	For use in...
Proteinase K (Vial 2)	Dissolve Proteinase K in 4.5 ml Elution Buffer.	Store in aliquots at −15 to −25°C for 12 months.	RNA isolation protocol, Step 1.
Wash Buffer I (Vial 4)	Add 20 ml absolute ethanol to Wash Buffer I. ⚠ ***Label and date bottle after adding ethanol.***	Store at +15 to +25°C until the expiration date printed on the kit label.	RNA isolation protocol, Step 7.
Wash Buffer II (Vial 5)	Add 40 ml absolute ethanol to Wash Buffer II. ⚠ ***Label and date bottle after adding ethanol.***	Store at +15 to +25°C until the expiration date printed on the kit label.	RNA isolation protocol, Step 8.
DNase I (Vial 6)	Dissolve DNase I in 2 ml Elution Buffer and mix thoroughly.	Store in aliquots at −15 to −25°C for 12 months.	RNA isolation protocol, Step 6.

2.2. Protocols

Experimental overview



Isolation of RNA from formalin-fixed, paraffin-embedded tissue

Deparaffinization

The following steps describe the deparaffinization procedure.

1. For one, 1 to 10 µm section of formalin-fixed, paraffin-embedded tissue in a 1.5 ml reaction tube, add 800 µl Hemo-De or Xylene.
   – Vortex for at least 4 seconds three times.
   – Incubate for 2 minutes.
   – Vortex at least 4 seconds three times.
   – Incubate for 5 minutes.
   – Centrifuge for 2 minutes at maximum speed (12,000 to 14,000 × *g*).
   – Discard supernatant by aspiration.

   ⚠ ***Directly cap the tubes to avoid drying of tissue sections.***

   ⚠ ***If the tissue appears to be floating in the Xylene, repeat spinning for 2 minutes.***

2. Repeat Step 1.

3. Add 800 µl absolute ethanol, flick the tube to dislodge the pellet, and vortex for 4 seconds three times.
   – Centrifuge for 2 minutes at maximum speed (12,000 to 14,000 × *g*).
   – Discard supernatant by aspiration.

4. Add 800 µl 70% ethanol, flick the tube to dislodge the pellet, and vortex for 4 seconds three times.
   – Centrifuge for 2 minutes at maximum speed.
   – Discard supernatant by aspiration.

5. After removal of 70% ethanol, respin the tube for 10 to 20 seconds and carefully remove the residual fluid with a fine-bore pipette.
   – Incubate the open tubes in a heating block for 5 to 15 minutes at +55°C to air-dry the tissue pellet.
   – Proceed with Step 1 of the RNA isolation protocol.

Alternative deparaffinization

The following steps describe the alternative deparaffinization procedure.

1. For one, 1 to 10 µm section of formalin-fixed, paraffin-embedded tissue on a microscope slide, place the slide in a Hemo-De or Xylene bath and incubate for 10 minutes.

2. Tap off excess liquid and place the slide into absolute ethanol for 10 minutes.

3. Change bath and incubate the slide for an additional 10 minutes in absolute ethanol.

4. Scratch the deparaffinized section from the slide by using a sterile single-use scalpel and place it in a 1.5 ml reaction tube.

   ⚠ ***To avoid scattering of the tissue, scratch the section from the microscope slide before it has dried.***

5. Dry the tissue for 10 minutes at +55°C.
   – Proceed with Step 1 of the RNA isolation protocol.

RNA isolation protocol

The following protocol describes the RNA isolation for one, 1 to 10 µm section of formalin-fixed, paraffin-embedded tissue on a microscope slide.

If necessary, multiple preparations can be pooled after Step 4.

1. To one tissue pellet, deparaffinized as described above, add 60 µl Tissue Lysis Buffer (Bottle 1) and 10 µl 10% SDS*.
   – Vortex 3 × 4 seconds, spin down, and add 30 µl Proteinase K working solution.
   – Vortex 3 × 4 seconds, spin down, and incubate for 3 hours at +55°C.

2. Add 200 µl Binding Buffer (Bottle 3) and 200 µl absolute ethanol.
   – Vortex 3 × 4 seconds and spin down.

3. Combine the High Pure Filter Tube with a Collection Tube and pipette the lysate into the upper reservoir.

4. Centrifuge for 30 seconds at 8,000 × *g* in a microcentrifuge and discard the flow through.
   Steps 3 to 4 can be repeated in order to load the column with additional sample material; do not overload the column.

5. Centrifuge for 1 minute at maximum speed to dry filter.

6. Add 30 µl DNase Solution (3 µl DNase Incubation Buffer and 27 µl DNase).
   – Incubate for 15 minutes at +15 to +25°C.

7. Add 300 µl Wash Buffer I working solution (Bottle 4) to the upper reservoir.
   – Centrifuge for 15 seconds at 8,000 × *g*; discard the flow through.

8. Add 300 µl Wash Buffer II working solution (Bottle 5).
   – Centrifuge for 15 seconds at 8,000 × *g*; discard the flow through.

9. Add 200 µl Wash Buffer II working solution (Bottle 5).
   – Centrifuge for 15 seconds at 8,000 × *g*; discard the Collection Tube.

10. Place the High Pure Micro Filter Tube in a fresh Collection Tube and centrifuge for 2 minutes at maximum speed.

11. Place the High Pure Micro Filter Tube in a fresh 1.5 ml reaction tube.
    – Add 20 µl Elution Buffer (Bottle 8).
    – Incubate for 1 minute at +15 to +25°C.
    – Centrifuge for 1 minute at 8,000 × *g*.

12. Reload the Eluate; incubate for 1 minute at +15 to +25°C.
    – Centrifuge at 8,000 × *g* for 1 minute.

13. The microcentrifuge tube now contains the eluted RNA.
    – Use 0.5 to 5 µl of the eluted RNA directly in RT-PCR or store the eluted RNA at −80°C for later analysis.

⚠ ***Before photometric determination of the RNA concentration, centrifuge the eluate for 2 minutes at maximum speed and transfer supernatant to a fresh 1.5 ml reaction tube without disturbing glass fibers at the bottom of the original tube.***

3. Results

Purified RNA is free of DNA, nucleases, and all cellular and sample components that interfere with RT-PCR. Typically, RNA fragments isolated from formalin-fixed tissue ranged from 150 up to 1,500 bases.

Recovery with respect to elution volume

Elution volume [µl]	Recovery [%]
40	84
20	80
10	83

Five micrograms of total RNA were used for this recovery test.

Fragment length distribution

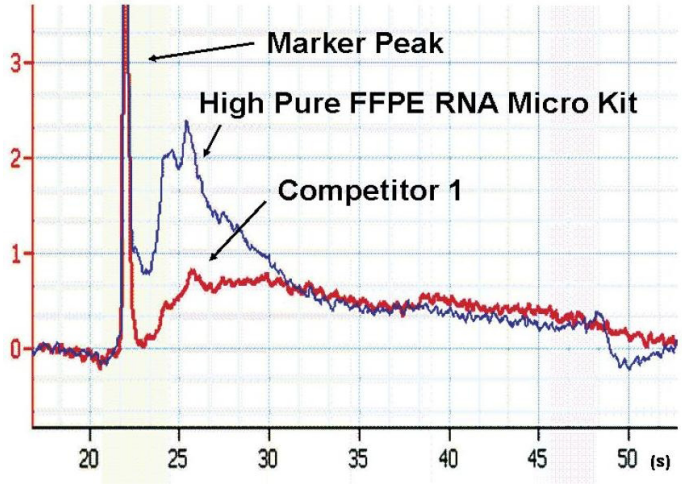


Fig. 1: Electropherogram (Bioanalyzer, Agilent) of isolated RNA from a 5 µm slice of an FFPE breast tumor sample.The High Pure FFPE RNA Micro Kit is optimized for FFPE sample material shown by the high recovery of even small RNA fragments.

Performance in RT-PCR

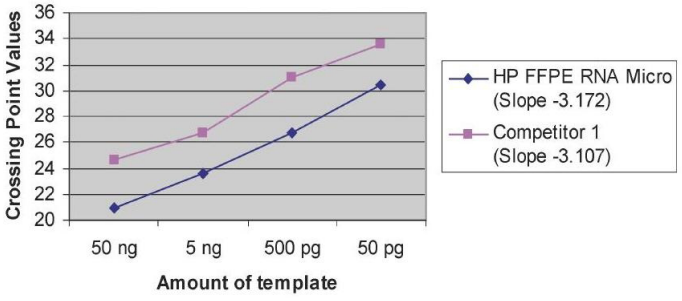


Fig. 2: RNA was isolated from a 5 µm slice of an FFPE breast tumor sample using either the High Pure FFPE RNA Micro Kit or a competitor product. The RNA was then used as template in a LightCycler® 2.0 System RT-PCR assay targeting ß2-microglobulin utilizing the LigthCycler® RNA Amplification Kit SYBR Green ITemplate RNA isolated using the High Pure FFPE RNA Micro Kit shows very good performance, that is, high sensitivity shown by early crossing point values and linearity shown by consistent slope values in LightCycler® 2.0 System RT-PCR assays.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low RNA yield or purity.	Kit stored under non-optimal conditions.	Store kit at +15 to +25°C at all times.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	Store all buffers at +15 to +25°C.
		Close all reagent bottles tightly after each use to preserve pH, stability, and to avoid contamination.
		After reconstituting lyophilizates, aliquot and store at −15 to −25°C.
	Ethanol not added to Wash Buffer.	Add absolute ethanol to the buffers before using.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Always label Wash Buffer vials to indicate if ethanol has been added.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
	Ethanol not added to the lysate in Step 3.	Addition of 0.5 volume of absolute ethanol to the lysate is necessary to promote selective binding of RNA to the glass fibers.
	High levels of RNase activity.	Be sure to create an RNase-free working environment.
		Process starting material immediately or store it at −80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store it immediately at −80°C.
Tissue homogenate is viscous and difficult to pipette; low RNA yield.	Insufficient disruption or homogenization.	Add 350 µl of Lysis/Binding Buffer and repeat homogenization step to reduce viscosity.
Low yield and/or poor performance in RT-PCR.	Nucleic acid is crosslinked to impurities.	Increase Proteinase K digestion time in Step 1 of the RNA isolation protocol to 16 hours to overnight.
	Too much starting material.	Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.
Clogged filter tube.	Insufficient disruption and/or homogenization.	Example: increase the disruption time for the rotor-stator homogenizer or pass through the syringe/needle additional times.
	Too much starting material.	Reduce amount of starting material and/or increase the amount of Lysis/Binding Buffer.
Absorbance (A_{260} nm) reading of product too high.	Glass fibers, which might co-elute with nucleic acid, scatter light.	▪ Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 2 minutes at maximum speed. ▪ Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Samples pop out of wells in agarose gels.	Eluate contains ethanol (from the Wash Buffer).	▪ After the wash step, do not let the flow through touch the bottom of the High Pure Filter Tube. ▪ Empty collection tube, reinsert filter tube in emptied collection tube, and recentrifuge for 30 seconds.

5. Additional Information on this Product

5.1. Test Principle

How this product works

To prepare tissue sections for RNA isolation, fixation reagents must be removed from the samples; after deparaffinization, the sections are ready for use with the High Pure FFPE RNA Micro Kit.

① The deparaffinized tissue samples are disrupted and homogenized during incubation with Proteinase K.

② In the presence of chaotropic salts, nucleic acids bind specifically to the surface of glass fibers that are prepacked in the kit's High Pure Micro Filter Tubes.
– The binding reaction occurs within seconds due to the disruption of the organized structure of water molecules and interaction with nucleic acids.
– The binding process is specific for nucleic acids in general, but the binding conditions are optimized for RNA.

③ Bound RNA is purified in a series of rapid wash-and-spin steps to remove unwanted cellular components.

④ To eliminate residual DNA, an on-column digestion with DNase I is performed after nucleic acid binding to the column.

⑤ Subsequently, a low-salt elution releases the RNA from the glass fibers in a minimum volume of 10 µl.

The process does not require RNA precipitation or organic solvent extractions.

5.2. Quality Control

Formalin-fixed, paraffin-embedded tissue sections are homogenized by overnight Proteinase K digestion and purified as described. RNA yield is determined by measuring the optical density at 260 nm. The RNA eluate and specific primers for the β2M gene are used in one-step RT-PCR. In the following PCR using the LightCycler® System, performed with the LightCycler® RNA Amplification Kit SYBR Green I and specific primers for β2M, the expected amplification signal is obtained at a Cp value <24. Absence of contaminating genomic DNA is examined by PCR using the LightCycler® System without a reverse transcriptase step; no amplification product is obtained.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Update of quality control.
Editorial changes.

6.3. Trademarks

HIGH PURE and LIGHTCYCLER are trademarks of Roche.
SYBR is a trademark of Thermo Fisher Scientific Inc..
All other product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of LifeScience products.

6.5. Regulatory Disclaimer

For general laboratory use.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country to display country-specific contact information.

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